BUNDESREPUBLIK DEUTSCHLAND DEUTSCHES PATENT- UND MARKENAMT	**Patent Application Publication DE 197 21 211 A 1** File Reference: 197 21 211.5 Filing Date: May 21, 1997 Publication Date: November 26, 1998	Int. CL6: **A 61 K 45/06** A 61 K31/70 A 61 K31/40 A61 K 51/00

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The following information is published in the version submitted by the applicant

Search Request according to paragraph 43, section first sentence PatG has been filed

Combination of anti-inflammatory substances with immune stimulating agents and cytotoxic agents for the treatment of tumors

The invention relates to a method of treating tumors using a triple combination therapy. The components of said triple combination therapy consist of an anti-inflammatory therapy component (e.g. indometacin, diclofenac, dexamethasone), a therapy component stimulating the immune system (e.g. interleukin-2) and a tumor damaging therapy component (e.g. cystostatics/cystotoxic substances, radioactive radiation or thermal therapy).

Description

Introduction

In almost any clinically apparent tumor an infiltration of immune system cells and especially of lymphocytes can be demonstrated (8, 12, 13, 14, 15). In vitro experimental approaches have shown that those tumor infiltrating lymphocytes (TIL) are able to recognize and destroy specific tumor cells (8, 13). From most patients with tumors it is possible to isolate lymphocytes or so-called natural killer cells (NK) from the peripheral blood which in in vitro assay systems have the potential to specifically recognize and destroy tumors (8, 13, 14).

With this research it was shown that, apparently, against any tumor, there exists also an immune reaction directed against this tumor. For unknown reasons, however, in a clinical setting an immune reaction leading to therapeutic success can be provoked only in few cases and with a low success rate (7, 12, 13, 14, 15).

Advantages of Immunotherapy against Tumors

In principal immune reactions specifically directed against a tumor offer the possibility to specifically eliminate the tumor while saving healthy tissue. Thus the disadvantages of surgical, radiologic or chemotherapy intervention are avoided which always involve damage to the healthy tissue. Therefore a specific anti-tumor reaction of the immune system seems to be the only possibile way of intervention for up to now incurable/inoperable tumors (e.g. metastasis, dimensions too large, vital sensitive organs directly adjacent to the tumor).

Previous Immunotherapeutic Treatment Concepts

Thus there has been no lack of attempts to make use of these specific anti-tumor reactions of lymphocytes and NK cells to cause the elimination of tumors. To this effect either the immune system of the cancer patient was stimulated directly (e.g. by intravenous administration of interleukin-2 or interferon-gamma) or cells from the immune system of the cancer patient were isolated from the tumor or the peripheral blood respectively and propagated in vitro. Subsequently those cells of the immune system with a verifiable specific anti-tumor reactivity were re-infused into the patient (13, 14). Almost none of the expectations of the immune therapy experiments were fulfilled. There were either no or only insignificant therapeutic effects. One of the few exceptions is the therapy of the kidney carcinoma with interleukin-2. But even in this case only response rates of 30% at most and curative therapy results in 5 to 10% of the treated cases were achieved (7). So far there is therefore no immunotherapeutic experimental approach which has shown satisfying clinical effects.

Because of the lack of clinical results, efforts have been made to increase the effectiveness by combining the immunotherapy with a surgical intervention, radiation or chemotherapy (1, 2). In appropriate model systems the effectiveness of immunotherapy by combining it with radiation or chemotherapy can be decisively increased in animal experiments (7). Unfortunately it became obvious that even for these combination therapies the expected therapy results are not achieved in a clinical setting.

Problem

The existing problem is characterized by the lack of a therapy method for the treatment of cancer providing reliable effectiveness and good tolerance.

Approach to Problem Solving

In connection with radiation therapy or chemotherapy, inflammatory reactions can be observed (11). These inflammatory reactions can lead to an increase of the immune reaction directed against the tumor. In our own experimental investigations we attempted to clarify to what degree inflammatory processes occurring in connection with radiation therapy and chemotherapy contribute to an increase of effectiveness of immunostimulatory substances. In our experiments interleukin-2 was chosen as representative for the heterogenous group of the immune stimulating substances because interleukin-2 shows a certain therapeutic effect in a clinical setting when treating kidney carcinoma. In our experiments we first used the anti-inflammatory agent indomethacin and later other anti-phlogistics such as aspirin, diclofenac, dexamethasone, naproxen, piroxicam and ibuprofen. Since cytostatics as well as radiation therapy represent more or less specific noxas for tumors, we additionally investigated whether the noxa hyperthermia was also suitable for the combination with anti-inflammatory substances und stimulators of the immune system for the treatment of tumors.

The initial assumption was that the synergetic effects of immune stimulating therapy and radiation therapy, hyperthermia and chemotherapy respectively were reduced by administration of indomethacin.

Problem Solving and Experimental Findings

To our surprise the anti-tumor effect of combined chemo-immunotherapy or radiation-immunotherapy respectively was potentiated multiple times by combining it with several different types of anti-phlogistics.

An other surprising finding shows that the triple combination therapy used by us achieved very good effects even at low

dosage of radiation or cytostatics respectively (Appendix: Example 4, 10). In contrast to this, dual combination therapies and monotherapies hardly showed any effect, even with much higher radiation dosages.

According to our investigations hyperthermia is also suitable for combination therapy with anti-inflammatory substances (e.g. indomethacin) and immune stimulants (e.g. interleukin-2) (Appendix: Example 11).

When anti-phlogistic therapy was started only shortly before radiation therapy or after the radiation therapy, no or only considerably reduced synergistic effects could be observed. (Appendix: Example 5). However, when the anti-phlogistics were administered for a duration of 3 days before the radiation therapy, and stopped one day before radiation, considerable synergistic effects could still be observed. (Appendix: Example 5). Since the anti-phlogistics used have a half life of only a few hours (16) the simultaneous administration of anti-phlogistics and interleukin-2 has no significance for the observed effects. This observation implies that the anti-phlogistics do not lead directly to an increase of effectiveness of radiation-immunotherapy but that changes are caused by the administration of anti-phlogistics which enable the effectiveness of immunotherapy in combination with radiation therapy. A combination of immunotherapy only with the administration of anti-phlogistics had no therapeutic effects and radiation (chemotherapy, hyperthermia) immunotherapy alone also had no effects at all. (Appendix: Examples 2, 3, 4, 8, 9, 10, 11, 15 12). A combination of radiation-immunotherapy with anti-phlogistics being administered not earlier than radiation also didn't show any improvements of the therapeutic outcome (Appendix: Example 5). This indicates that by pre-treatment with anti-inflammatory substances a situation is created where the synergistic effects of radiation immunotherapy can be created. Corresponding findings were shown for the combination therapy of cytostatics with anti-inflammatory substances and immune stimulants (Appendix: Example 10).

Advantages of the New Triple Combination Therapy

Tumors which are not treatable with chemotherapy, hyperthermia or radiation therapy as monotherapies and in dual combination therapy with immune stimulants or anti-inflammatory medications can be successfully treated with the new triple combination therapy. Treatment success can be achieved already at low radiation and chemotherapy doses. Therefore less side effects in comparison with high dosed radiation or chemotherapy can be expected.

State of the Art and Comparison with Our Own Investigation Results

The state of the art in immunotherapy of tumors consists of monotherapy with immunostimulatory substances and the combination of immune stimulating substances with surgical removal, radiation, hyperthermia and chemotherapeutical treatment of tumors (1, 2, 3). Furthermore, in clinical settings experimental therapies are conducted where immune defense cells of the cancer patient, activated and propagated ex vivo, are re-infused into the patient. In addition to these re-infused cells the patients receive a treatment with immunostimulatory substances (12, 13, 14).

A combination of interleukin-2 with the anti-phlogistic indomethacin was already described (4). However, the effect described above is, according to the literature, a synergy of interleukin-2 with the prostaglandin synthesis suppressed by indomethacin. Here interleukin-2 was given at the same time as indomethacin (4). In our experiments, on the other hand, the positive synergistic effect could still be observed when the anti-phlogistics therapy was completed already one day before radiation and the administration of interleukin-2 was started only after radiation (Appendix: Example 5). Because in our experiments no anti-phlogistic was administered during administration of interleukin-2, the effects described by us are not a direct interaction between the anti-phlogistic (prostaglandin synthesis inhibitors) indomethacin and interleukin-2 as described under (4), but a manipulation of the tumor before radiation therapy, whereby the tumor, after radiation therapy, becomes sensitive to immune therapy directed against the tumor.

The advantages of the combination of radiation therapy with interleukin-2 treatment are already described in the literature (6, 9). The advantageous effect was here considered to be caused by an increase of antigenicity of the tumor and a slow down in tumor growth (9). However, our experiments did not show any effectiveness of the dual combination radiation and administration of interleukin-2 (Appendix: Example 2). Only when an anti-phlogistic was administered before radiation therapy, positive anti tumor effects could be observed (Appendix: Example 4, 5). It will be appreciated that the mechanisms of synergy of radiation therapy with immunostimulatory therapy described in the literature have no impact on the therapy successes observed by us.

In the positive interactions of cytostatics with immunotherapy already described, the positive effect of the combination is attributed to a decrease of the immune suppressing effects of the cytostatic therapy by immune stimulants (2). In our experiments the positive effects of the administration of interleukin-2 were observed only after pre-treatment with the anti-phlogistic indomethacin (Appendix: Example 10). An immune supression evoked by radiation therapy or cytostatic therapy should be antagonisable even without the pre-treatment with anti-phlogistics by administering interleukin-2. In spite of that we were not able to observe any effects in our experiments by only administering radiation and immune stimulation with interleukin-2. (Appendix: Example 2,3). Therefore the effects of combination therapy observed by us can not be contributed to the effects of a combination of cytostatics with immune stimulants already described. As described in Appendix 8 the immune stimulator interleukin-2 can also be replaced by other immune stimulating principles as the bacterial lipopolysaccaride from E .coli.

Generally it can be said that the triple combination therapy is effective even against established tumors, shown until day 17 after tumor application (Appendix: Example 7). At that time dual combination therapies or monotherapies are not effective anymore, even on day 10 after tumor injection they did not show any further effect (Appendix: Examples 2, 3, 8, 9, 10, 11, 12).

Overall it can be said that the advantageous effectiveness of the triple combination of medications with anti-phlogistic

effects and immune stimulating substances and radiation therapy, hyperthermia or chemotherapy has not yet been described in form of a triple combination therapy.

Object of the Invention

Object of the invention is a combination of anti-inflammatory agents with a therapy component stimulating the immune system and a tumor damaging therapy component. Essential for the objective of the invention is a time dependent connection between the beginning of each therapy component: Administration of the anti-inflammatory agents is to begin before starting the tumor damaging therapy component. The administration of the anti-inflammatory agents can be continued after applying the tumor damaging therapy component. The application of the immune stimulating therapy component needs to occur, at the latest, after the application of the tumor damaging therapy component at the latest. It is possible, however, to begin with the immune stimulating therapy component already before starting the tumor damaging therapy component.

The anti-inflammatory agents can be the following or similar anti-inflammatory substances: indomethacin, acetylsalicylic acid, diclofenac, dexamethasone, ibuprofen, naproxen, salicylic acid, mefenamic acid, flutenamic acid, fenoprofen, metamizole, phenazone, aminophenazone, propyphenazone, phenylbutazone, oxyphenbutazone, corticoide, anti-inflammatory cytokines as interleukin-10 and TGF-β, soluble interleukin-1 receptors, interleukin-1 receptor antagonists, interleukin-l-converting enzyme inhibitors, NFκB inhibitors and other substances with anti-inflammatory effects.

The immunostimulatory therapy component can be one of the following or a similar therapy principle stimulating the immune system: e.g. interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interferon-α, interferon β, interferon-γ[1], TNF-α, vaccines, preparations of bacterial, viral or other pathogens, concanavalin A, antibodies for the activation of cells of the immune system, lectines, pyrogens, immune stimulating protein and/or carbohydrate sequences, from the outside introduced cells with immune stimulating effect.

The tumor damaging therapy component can be one of the following or a similar therapy principle: cyclophosphamide, chlorambucil, melphalan, busulfan, thiotepa, ifosfamide, methotrexate, 5-fluoruracil, mercaptopurine, vinblastine, vincristine, etoposide, teniposide, doxorubicin, daunorubicin, dactionmycin and other substances with cytotoxic and cytostatic effect, application of those tumor damaging agents with methods allowing the selective release of such substances inside of the tumor, radiation therapy of the tumor with α, β, γ or other tumor damaging radiation, local tumor specific thermal therapy, systemic thermal therapy.

This invention does explicitly not include any monotherapies or dual combination therapies of the single therapy components described above.

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[1] Note by the translator: The German term 'Interferon-y' is most likely an error

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Appendix

Examples proving the invention

Materials and Methods

Animals

4 to 6 week old female C57BL/6 mice were kept in groups of 5 animals per cage. The animals had free access to drinking water and standard mice food. The animals were kept according to SPF conditions and at a day-night cycle of 12 hours.

Tumors

The B16 melanoma (10) and the MCA 105 sarcoma (18), a sarcoma induced by methylcholantrene, were passaged subcutanuously in C57BL/6 mice (syngenic system). From these solid tumors a singel cell suspension was obtained by digestion with 0.4 mg/ml collagenase type IV (Sigma Chemical Co., St. Louis, MO, USA.) in RPMI 1640 culture medium, which in addition contained 100 U/ml penicillin G, 100 µg/ml streptomycin, 50 µg/ml gentamycin and 10 mM HEPES buffer. This suspension was passaged through a fine mesh wire net and washed with Hank's Balanced Salt Solution (HBSS). The number of vital cells was determined with the eosin staining technique. For in vivo experiments the number of vital cells was set at 2x105/ml in HBSS. 0.5 ml corresponding to 10^5 cells were injected into the C57BL/6 mice via the tail vein.

Therapy Methods

Radiation therapy was conducted with a Co-60γ radiation source. The intensity of the radiation source was 83 rad/minute. Before radiation the animals were anesthezised with Rompun / Ketavet and then exposed to the radiation source for a precalculated amont of time.

For thermal therapy (hyperthermia) the mice were anesthezised with 50 mg/kg Nembutal and exposed to a temperature of 41°C in a water bath in 50ml centrifuge tubes.

Acetylsalicylic acid, diclofenac, dexamethasone, indomethacin, ibuprofen, piroxicam and naproxen (Sigma Chemical Co., St. Louis, MO, U.S.A.) were dissolved in 1% carboxymethycellulose and applied by gavage.

Bacterial lipopolysaccaride (LPS) from Escherichia Coli (Sigma) was dissolved in 0.9% NaCl solution. A stock solution was produced and the biological effect on the C57BL/6 mice was tested. For therapeutic application a 1:20 dilution of the LD 50 dosage was used. The application was carried out intravenously; recombinant human interleukin-2 (Chiron) was diluted in 0.9 NaCl and administered twice a day at a dose of 100 000 units. Doxorubicin, methotrexate and cyclophosphamide (Sigma) were dissolved in 0.9% NaCl and injected into the tail vein.

Observation Parameters

As success parameter for the therapy the survival time of the animals is used. After completion of the experiment or after the death of the animals, an autopsy was conducted to determine the cause of death (tumor or other causes).

Statistics

For calculating the statistical significance of the experiment results the Student's T-Test was used. For animals surviving until completion of the experiment a survival period corresponding to the observation period was set for statistical evaluation.

Example 1

Influence of different radiation doses on the development of the B16 melanoma:
Result: The survival period of the animals carrying B6 is not prolonged by radiation[2] therapy alone.

Table 1a

Group	Group Size	Interleukin-2	Radiation Therapy Dose [rad] / Treatment Day	Indomethacin	Number of Tumor Cells Administered (B16-Melanoma)
I	5	-	0	-	100000
II	5	-	100 / day 10	-	100000
III	5	-	250 / day 10	-	100000
IV	5	-	500 / day 10	-	100000
V	5	-	750 / day 10		100000
VI	5	-	1000 / day 10	-	100000

Table 1b

Group	Survival Time [Days]	Survival Time: Average ± Standard Deviation	Statistics [T-Test versus Group I]
I	24;25;27;26;25;	25.4 ± 1.0	
II	24;25;26;27;27;	25.8 ± 1.2	p>0.05
III	24;26;27;28;24;	25.8 ± 1.6	p>0.05
IV	28;27;26;24;28;	26.6 ±1.5	p>0.05
V	25;27;27;24;29;	26.4 ± 1.8	p>0.05
VI	17;23;19;22;20:	20.2 ±2.1	p> 0.01

Example 2
Influence of the combination of interleukin-2 (twice a day 100 000 U/kg) with radiation therapy on the B16 melanoma

Result
The combination of radiation therapy with administration of interleukin-2 has no influence on the survival of mice carrying B16 melanoma.

[2] Note by the translator: The German term ‘Suahientherapie is an error. It is not a German word. Most likely it is ‘Strahlentherapy’ (radiation therapy)

Table 2a

Group	Group Size	Interleukin-2 [twice a day 100000U/kg]	Radiation Therapy Dose [rad] / Treatment Day	Indomethacin	Number of Tumor Cells Administered (B16-Melanoma)
I	5	day 11 to 17	0	-	100000
II	5	day 11 to 17	100 /day 10	-	100000
III	5	day 11 to 17	250 / day 10	-	100000
IV	5	day 11 to 17	500 / day 10	-	100000
V	5	day 11 to 17	750 /day 10	-	100000
VI	5	day 11 to 17	1000 / day 10	-	100000

Table 2b

Group	Survival Time [Days]	Survival Time: Average ± Standard Deviation	Statistics [T-Test versus Group 1]
I	24, 24, 27, 27, 24	25.2 ± 1.5	
II	25, 25, 24, 27, 27	25.6 ± 1.2	$p>0.05$
III	24, 23, 28, 26, 24	25.0 ± 1.8	$p>0.05$
IV	25, 23, 26, 24, 28	25.2 ± 1.7	$p>0.05$
V	24, 24, 25, 27, 26	25.2 ± 1.2	$p>0.05$
VI	22, 18, 19, 19, 20	19.6 ± 1.4	$p<0.005$

Example 3

Influence of the combination of indomethacin (4 mg/kg/day) (day 5-11) with radiation therapy on the development of the B16 melanoma.

Result

Neither radiation therapy nor the combination of radiation therapy with 4mg/kg indomethacin show a significant effect on the development of the B16 melanoma.

Table 3a

Group	Group Size	Interleukin-2 [IU/day]	Radiation Therapy Dose [rad] / Treatment Day	Indomethacin [4mg/kg]	Number of Tumor Cells Administered (B16-Melanoma)
I	5	-	0	day 5- day 11	100000
II	5	-	100 / day 10	day 5- day 11	100000
III	5	-	250 day 10	day 5- day 11	100000
IV	5	-	500 / day 10	day 5- day 11	100000
V	5	-	750 / day 10	day 5- day 11	100000
VI	5	-	1000 /day 10	day 5- day 11	100000

Table 3b

Group	Survival Time [Days]	Survival Time: Average ± Standard Deviation	Statistics [T-Test versus Group 1]
I	23, 25, 24, 27, 25	24.8 ± 1.3	
II	24, 26, 24, 23, 27	24.8 ± 1.5	$p>0.05$
III	25, 24, 28, 26, 25	25.6 ± 1.4	$p>0.05$
IV	27, 27, 24, 27, 28	26.6 ± 1,4	$p>0.05$
V	24, 30, 27, 25, 30	27.2 ± 2.5	$p>0.05$
VI	17, 19, 19, 21, 20	19.2 ± 1.3	$p<0.005$

Example 4

Influence of the triple combination therapy consisting of radiation therapy on day 10, a therapy with indomethacin at 4 mg/kg/day from day 6 to day 11 and the administration of interleukin-2.

Result

A significant increase in the survival time of the animals was observed only from a radiation dose of 250 rad on. At 500 and 750 rad radiation none of the animals died during the observation period of 50 days. At autopsy of those animals (group IV and V) no tumor in the lung could be determined. In animals radiated with 1000 rad no tumor in the lung could be determined after their death as well.

Conclusion

By means of combination therapy of 250 to 750 rad radiation with administration of indomethacin und interleukin-2 the survival of the animals could be prolonged significantly.

Table 4a

Group	Group Size	Interleukin-2 [twice a day 100000U/kg]	Radiation Therapy Dose [rad] / Treatment Day	Indomethacin	Number of Tumor Cells Administered (B16-Melanoma)
I	10	day 11 to 17	0	day 5- day 11	100000
II	10	day 11 to 17	100 / day 10	day 5- day 11	100000
III	10	day 11 to 17	250 / day 10	day 5- day 11	100000
IV	10	day 11 to 17	500 /day 10	day 5- day 11	100000
V	10	day 11 to 17	750 / day 10	day 5- day 11	100000
VI	10	day 11 to 17	1000 / day 10	day 5- day 11	100000

Table 4b

Group	Survival Time [Days]	Survival Time: Average ± Standard Deviation	Statistics [T-Test versus Group 1]
I	24, 25, 24, 27, 25, 26, 27, 23, 24, 26	25.1 ± 1.3	
II	23, 25, 27, 24, 27, 23, 26, 25, 26, 24	25.0 ± 1.4	$p>0.05$
III	25, 33, 26, 27, 33, 32, 34, 27, 32, 34	30.3 ± 3.4	$p<0.001$
IV	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50	> 50	$p<0.001$
V	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50	> 50	$p<0.001$
VI	15, 19, 19, 21, 20, 17, 22, 16, 18, 15	18.2 ± 2.3	$p<0.001$

Example 5

Influence of the indomethacin therapy duration on the therapy results of the triple combination

Result

The triple combination therapy showed positive results only when indomethacin therapy was started before radiation therapy and no big time gap existed between the end of the indomethacin therapy and the beginning of the radiation therapy.

Table 5a

Group	Group Size	Interleukin-2 [twice per day 100000U/kg]	Radiation Therapy Dose [rad] / Treatment Day	Indomethacin [4mg/kg/day]	Number of Tumor Cells Administered (B16-Melanoma)
I	10	day 11 day 17	500 / Tag 10	-	100000
II	10	day 11 day 17	500 / Tag 10	day 5- day 9	100000
III	10	day 11 to 17	500 / day 10	day 5- day 17	100000
IV	10	day 11 to 17	500 / day 10	day 10 - day 17	100000
V	10	day 11 to 17	500 / day 10	day 10	100000
VI	10	day 11 to 17	500 /day 10	day 10 - day 17	100000
VII	10	day 11 to 17	500 / day 10	day 5- day 8	100000
VIII	10	Tag 11 to 17	500 / day 10	day 4 - day 8	100000

Table 5b

Group	Survival Time [Days]	Survival Time: Standard Deviation Average ±	Statistics [T-Test versus Group I]
I	25, 25, 24, 26, 25, 24, 27, 24, 23, 26	24.9 ± 1.1	
II	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,>50	> 50	$p<0.001$
III	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,>50	> 50	$p<0.001$
IV	25, 23, 26, 23, 27, 26, 27, 24, 26, 24	25.1 ± 1.4	$p>0.05$
V	24, 24, 26, 26, 25, 26, 27, 23, 26, 25	25.2 ± 1.2	$p>0.05$
VI	23, 24, 27, 25, 26, 27, 27, 23, 27, 24	25.3 ± 1.6	$p>0.05$
VII	24, 26, 27, 30, 26, 25, 27, 24, 27, 24	26.0 ± 1.8	$p>0.05$
VIII	26, 30, 28, 24, 26, 24, 23, 26, 28, 28	26.3 ± 2.1	$p>0.05$

Example 6

Influence of the interleukin-2 therapy duration on the therapy results of the triple combination therapy

Result

When interleukin-2 is administered before radiation therapy it diminishes the effectiveness of the triple combination therapy very significantly ($p<0.001$). If the duration of the therapy with interleukin-2 is shortened before radiation therapy a significant positive influence on the therapy results can be seen ($p<0.05$). Only if the administration of interleukin-2 is started after radiation the effectiveness of the triple combination therapy can develop fully.

Table 6a

Group	**Group Size**	**Interleukin-2 [twice per day 100000U1kg]**	**Radiation Therapy Dose [rad] / Treatment Day**	**Indomethacin [4mg/kg/day]**	**Number of Tumor Cells Administered (B16-Melanoma)**
I	10	-	500 / day 10	day 5- day 11	100000
II	10	day 5 to 9	500 / day 10	day 5- day 11	100000
III	10	day 5 to 10	500 / day 10	day 5- day 11	100000
IV	10	day 5 to 17	500 / day 10	day 5- day 11	100000
V	10	day 9 to 17	500 / day 10	day 5- day 11	100000
VI	10	day 10 to 17	500 / day 10	day 5 - day 11	100000
VI	10	day 11 to 17	500 / day 10	day 5- day 11	100000

Table 6b

Group	Survival Time [Days]	Survival Time: Average ± Standard Deviation	Statistics [T-Test versus Group I]
I	23, 25, 26, 26, 25, 23, 24, 25, 26, 25	24.8 ± 1.1	
II	24, 26, 25, 26, 27, 25, 24, 23, 23, 26	24.9 ± 1.3	$p>0.05$
III	23, 26, 23, 27, 24, 25, 26, 25, 23, 27	24.9 ± 1.5	$p>0.05$
IV	30, 33, 34, 30, 35, 34, 30, 26, 32, 35	31.9 ± 2.7	$p<0.01$
V	35, 34, 33, 37, 30, 36, 34, 33, 34, 37	34.3 ± 2.0	$p<0.01$
VI	>50, >50, >50, >50, >50, >50, >50, >50, >50,>50	> 50	$p<0.001$
VI	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50	> 50	$p<0.001$

Example 7

Influence of the time of radiation therapy on the therapy result

Result

The moment of radiation therapy is not important for the therapy results of the triple combination therapy. It is important, even if treatment is started late, that indomethacin is administered before radiation and that after radiation a treatment with interleukin-2 follows.

Table 7a

Group	Group Size	Interleukin-2 [twice per day 100000U/kg]	Radiation Therapy Dose [rad] / Treatment Day	Indomethacin [4mg/kg]	Number of Tumor Cells Administered (B16-Melanoma)
I	10	day 11 to 17	0	day 5- day 11	100000
II	10	day 11 to 17	500 / day 10	day 5- day 11	100000
III	10	day 11 to 17	500 / day 12	day 7 - day 13	100000
IV	10	day 11 to 17	500 / day 15	day 5- day 11	100000
V	10	day 16 to 22	500 l day 15	day 10 - day 16	100000
VI	10	day 18 to 24	500 / day 17	day 12 - day 18	100000

Table 7b

Group	Survival Time [Days]	Survival Time: Average ± Standard Deviation	Statistics [T-Test versus Group I]
I	25, 23, 26, 24, 27, 26, 23, 27, 26, 25	25.2 ± 1.4	
II	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50	> 50	p<0.001
III	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50	> 50	p<0.001
IV	27, 30, 26, 26, 24, 23, 24, 24, 26, 27	25.7 ± 2.0	p>0.05
V	>50, >50, >50, >50, >50, >50, >50, >50, >50,>50	> 50	p<0.001
VI	>50, >50, >50, >50, >50, >50, >50, >50, >50,>50	> 50	p<0.001

Example 8

Replacement of interleukin-2 (IL-2) with the nonspecific immune stimulant lipopolysaccaride from E. coli (LPS).

Result

Interleukin-2 can be replaced with LPS. Like interleukin-2 LPS is only effective in the triple combination therapy.

Table 8a

Group	Group Size	Immune stimul. IL-2 or LPS day 11 to 17	Radiation Therapy Dose [rad] / Treatment Day	Indomethacin [4mg/kg/day]	Number of Tumor Cells Administered (B16-Melanoma)
I	10	-	500 / day 10	day 5- day 11	100000
II	10	IL-2	500 / day 10	day 5- day 11	100000
III	10	LPS	500 / day 10	day 5- day 11	100000
IV	10	LPS	500 /day 10	-	100000
V	10	LPS	-	day 5- day 11	100000
V1	10	LPS	-	-	100000

Tab. 8b

Group	**Survival Time [Days]**	**Survival Time: Average ± Standard Deviation**	**Statistics [T-Test versus Group I]**
I	24, 22, 26, 25, 24, 23, 26, 25, 24, 24	24.3 ± 1.2	
II	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	> 50	p<0.001
III	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	> 50	p<0.001
IV	25, 26, 25, 27, 23, 24, 23, 26, 25, 26	25.0 ± 1.3	p>0.05
V	24, 23, 26, 25, 26, 24, 25, 23, 26, 27	24.9 ± 1.3	p>0.05
Vi	24, 25, 23, 24, 26, 27, 24, 23, 26, 25	24.7 ± 1.3	p>0.05

Example 9

Replacement of indomethacin (Indo.) [4 mg/kg] with acetylsalicylic acid (Ass.) [20 mg/kg], diclofenac (Dic.) [2 mg/kg], dexamethasone (Dex.) [1 mg/kg], piroxicam (Pir.) [1 mg/kg], naproxen (Nap.) [5 mg/kg] or ibuprofen (Ibu.)[3] [10 mg/kg].

Result

Indomethacin can be replaced with other medications with anti-inflammatory effect.

[3] Note by the translator: The German term '(1 bu.)' is most likely an error

Table 9a

Group	Group Size	Interleukin-2 [twice per day 100000 U/kg]	Radiation Therapy Dose [rad] / Treatment Day	Anti-phlogistic: Day 5 - Day 11	Number of Tumor Cells Administered (B16-Melanoma)
I	10	day 11 to 17	500 / Tag 10		100000
II	10	day 11 to 17	500 / Tag 10	Indo.	100000
III	10	day 11 to 17	500 / day 10	Ass.	100000
IV	10	day 11 to 17	500 /day 10	Dic.	100000
V	10	day 11 to 17	-	Dic.	100000
VI	10	-	500 / day 10	Dic.	100000
VII	10	-	-	Dic.	100000
VIII	10	day 11 to 17	500 / day 10	Pir.	100000
IX	10	day 11 to 17	500 / day 10	Nap.	100000
X	10	day 11 to 17	500 / day 10	Ibu.	100000
XI	10	day 11 to 17	500 / day 10	Dex.	100000

Table 9b

Group	Survival Time [Days]	Survival Time: Average Standard Deviation	Statistics [T-Test versus Group I]
I	23, 25, 25, 26, 23, 24, 26, 27, 26, 25	25.0 ± 1.3	
II	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	> 50	p<0.001
III	>50, >50, >50, >50, >50,>50, >50, >50, >50, >50,	> 50	p<0.001
IV	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	> 50	p<0.001
V	24, 23, 24, 27, 26, 24, 24, 27, 24, 26	24.9 ± 1.3	p>0.05
VI	26, 23, 25, 26, 26, 24, 25, 26, 23, 27	25.1 ± 1.3	p>0.05
VII	23, 24, 25, 27, 26, 27, 23, 25, 26, 25	25.1 ± 1.4	p>0.05
VIII	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	> 50	p<0.001
IX	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	> 50	p<0.001
X	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	> 50	p<0.001
XI	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	> 50	p<0.001

Example 10

Replacement of radiation (Rad.) [500 rad] with cytostatic therapy (cyclophosphamide (Cyclo.) [5 mg/kg], doxorubicin (Dox.) [5 mg/kg], methotrexat (MTX) [20 mg/kg].

Result

The radiation therapy in the concept of the triple combination therapy can be replaced with other cytostatics.

Table 10a

Group	Group Size	Interleukin-2 12x per day 100000U/kg]	Cytostatic /Cytotoxic Therapies am Tag 10	Indomethacin	Number of Tumor Cells Administered (B16-Melanoma)
I	10	-	-	-	100000
II	10	day 11 to 17	Rad.	day 5- day 11	100000
III	10	day 11 to 17	Cyclo.	day 5- day 11	100000
IV	10	day 11 to 17	Cyclo.	-	100000
V	10	-	Cyclo.	day 5- day 11	100000
VI	10	-	Cyclo.	-	100000
VII	10	day 11 to 17	Dox.	day 5- day 11	100000
VIII	10	day 11 to 17	MTX	day 5- day 11	100000

Table 10b

Group	Survival Time [Days]	Survival Time: Average ± Standard Deviation	Statistics [T-Test versus Group 1]
I	24, 23, 25, 26, 24, 23, 23, 26, 25, 25	24.4 ± 1.1	
II	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50	> 50	$p<0.001$
III	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50	> 50	$p<0.001$
IV	24, 25, 26, 23, 22, 23, 23, 25, 24, 27	24.2 ± 1.5	$p>0.05$
V	26, 27, 23, 24, 22, 27, 26, 24, 23, 27	24.9 ± 1.8	$p>0.05$
VI	22, 26, 25, 26, 27, 22, 24, 23, 23, 26	24.4 ± 1.7	$p>0.05$
VII	>50, >50, >50, >50, >50, >50, >50, >50, >50,>50	> 50	$p<0.001$
VIII	>50, >50, >50, >50, >50, >50, >50, >50, >50,>50	> 50	$p<0.001$

Example 11

Replacement of radiation with hyperthermia.

Result

Radiation therapy can be replaced by hyperthermia.

Table 11a

Group	Group Size	Interleukin-2 [twice per day 100000U/kg]	Hyperthermia 41°C, 30 min	Indomethacin	Number of Tumor Cells Administered (B16-Melanoma)
I	10	-	-	-	100000
II	10	-	day 10	-	100000
III	10	day 11 to 17	-	-	100000
N	10	day 11 to 17	day 10	day 5- day 11	100000
V	10	-	-	day 5- day 11	100000
VI	10	day 11 to 17	day 10	-	100000
VII	10	day 11 to 17	day 10	day 5- day 11	100000
VIII	10	-	day 10	day 5- day 11	100000

Tab. 11b

Group	Survival Time [Days]	Survival Time: Average ± Standard Deviation	Statistics [T-Test versus Group I]
I	22, 27, 24, 23, 25, 24, 26, 23, 27, 24	24.5 ± 1.6	
II	24, 27, 23, 27, 23, 23, 23, 27, 25, 26	24.8 ± 1.7	p>0.05
III	26, 27, 26, 26, 25, 24, 23, 23, 25, 27	25.2 ± 1.4	p>0.05
IV	>50, >50, >50, >50, >50, >50, >50, >50, >50,>50	> 50	p<0.001
V	24, 27, 22, 26, 25, 26, 24, 23, 23, 26	24.6 ± 1.6	p>0.05
VI	25, 25, 26, 24, 22, 25, 22, 23, 26, 27	24.5 ± 1.6	p>0.05
VII	23, 23, 27, 27, 25, 25, 26, 26, 22, 27	25.1 ± 1.8	p>0.05
VIII	23, 23, 26, 27, 22, 24, 23, 27, 26, 27	24.8 ± 1.9	p>0.05

Example 12

Transferability of the results attained for the B16 melanoma model to other tumors (MCA-103).

Result

The sarcoma induced by methylcholantrene reacts to triple combination therapy in the same way as the B16 melanoma.

Table 12a

Group	Group Size	Interleukin-2 twice per day 100000U/kg]	Radiation Therapy Dose [rad] / Treatment Day	Indomethacin	Number of Tumor Cells Administered (MCA-105)
I	10	day 11 to 17	500 /day 10	day 5- day 11	100000
II	10	day 11 to 17	-	-	100000
III	10	-	500 / day 10	-	100000
IV	10	-	-	day 5- day 11	100000
V	10	-	500 / day 10	day 5- day 11	100000
VI	10	day 11 to 17	-	day 5 - day 11	100000
VII	10	day 11 to 17	500 / day 10	-	100000
VIII	10	day 11 to 17	500 /day 10	day 5- day 11	100000

Table 12b

Group	Survival Time [Days]	Survival Time: Average ± Standard Deviation	Statistics [T-Test versus Group I]
I	30, 27, 29, 32, 33, 29, 28, 27, 33, 30	29.8 ± 2.1	
II	31, 27, 32, 29, 33, 32, 28, 27, 33, 31	30.3 ± 2.2	p>0.05
III	30, 33, 28, 33, 27, 32, 32, 30, 31, 27	30.3 ± 2.2	p>0.05
IV	28, 33, 33, 30, 28, 28, 27, 33, 34, 27	30.1 ± 2.7	p>0.05
V	30, 33, 33, 27, 27, 30, 31, 31, 29, 28	29.9 ± 2.1	p>0.05
VI	33, 28, 32, 27, 32, 32, 27, 28, 33, 31	30.3 ± 2.4	p>0.05
VII	33, 32, 32, 30, 27, 31, 30, 30, 28, 27	30.0 ± 2.0	p>0.05
VIII	>50, >50, >50, >50, >50, >50, >50, >50, >50, >50,	> 50	p<0.001

Patent claims

1. Combination of anti-inflammatory agents with immunostimulatory agents and tumor damaging agents (cytostatics, chemotherapeutics, physical measures) for treatment of tumors. 2. Combination of indomethacin with interleukin-2 and doxorubicin for treating tumors.

3. Combination of indomethacin (or acetylsalicylic acid, ibuprofen, diclofenac, dexamethasone, piroxicam, naproxen) with interleukin-2 (or LPS) and doxorubicin (or cyclophosphamide, methotrexate, γ-radiation[4], hyperthermia) for treating tumors.

4. Combination of anti-inflammatory agents with interleukin-2 (or LPS) and doxorubicin (or cyclophosphamide, methotrexate, γ-radiation[5], hyperthermia) for treating tumors.

[4] Note by the translator: The German term '7-Strahlung' is most likely an error

[5] Note by the translator: The German term '7-Strahlung' is most likely an error

5. Combination of indomethacin (or acetylsalicylic acid, diclofenac, dexamethasone, ibuprofen, piroxicam, naproxen) with immunostimulatory agents and Doxorubicin (or Cyclophosphamid, Methotrexat, γ-radiation[6], hyperthermia) for treating tumors.

6. Combination of anti-inflammatory agents with immunostimulatory agents and doxorubicin (or cyclophosphamide, methotrexate, γ-radiation, hyperthermia) for treating tumors.

7. Combination of indomethacin (or acetylsalicylic acid, diclofenac, dexamethasone, ibuprofen, piroxicam, naproxen) with immunostimulatory agents and tumor damaging agents (cytostatics, chemotherapeutics, physical measures) for treating tumors.

[6] Note by the translator: The German term '7-Strahlung' is most likely an error

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I, Martina Burkert, solemnly declare:

- That I am a translator of the German and English languages by profession, certified by the American Translators Association for English>German and an active member in good standing of the American Translators Association;

- That I am thoroughly conversant with these languages;

- That I have carefully rendered the translation from the German language into the English language of the following document:

Offenlegungsschrift DE 197 21 211 A 1
(Patent Application Publication DE 197 21 211 A 1)

- That the translation is, within generally accepted industry standards, a true and correct rendition of such document, in both meaning and context, to the best of my knowledge and belief.

M. Burkert

Translator

3-11-09

Date